

ary changes after the divergence of the striped bass and zebrafish lineages may be responsible for the differential partitioning of activities among the *Hox* PG2 genes in those divergent lineages.

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### Genetic analyses of adult pigment pattern development reveal homology and evolutionary novelty in *Danio* fishes

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Pigment patterns of *Danio* fishes are a convenient system for studying the evolution of development. In zebrafish, *D. rerio*, stripes form by migration and differentiation of distinct populations of melanophores: early metamorphic (EM) melanophores arise widely dispersed and then migrate into stripes, whereas late metamorphic (LM) melanophores arise already within stripes. EM melanophores require the kit receptor tyrosine kinase, as kit mutants lack these cells but retain LM melanophores that form a residual stripe pattern. To see if similar requirements are present in other species, we examined *D. albolineatus*, which has relatively few, uniformly dispersed melanophores. We isolated a null allele of *D. albolineatus* kit and asked whether residual, LM melanophores develop, as in *D. rerio*. We find that kit mutant *D. albolineatus* lack EM melanophores, yet retain LM melanophores. Interestingly, kit mutant *D. albolineatus* also develop a striped pattern similar to kit mutant *D. rerio*, indicating that (i) latent stripe-forming potential remains in this species, despite its uniform pattern; and (ii) evolutionary differences between *D. rerio* and *D. albolineatus* reflect changes in the behavior of kit-dependent melanophores, which migrate into stripes in *D. rerio* but fail to do so in *D. albolineatus*. Our results show how genetic analyses of closely related species can reveal both conservatism and innovation in developmental mechanisms, and the cellular processes underlying evolutionary changes in adult form.

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### Characterization of zebrafish Deltex homologues

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It is widely known that Deltex is a cytoplasmic protein that binds to Notch and may mediate CSL-independent Notch signaling. Deltex proteins share three functional domains: the WWE domain that binds to Notch ankyrin repeats, the Ring Finger (RF) domain that is often found in a subset of E3

ubiquitin ligases and a proline-rich region. We have cloned three full-length zebrafish *deltex* homologues, namely *deltex1*, *deltex2* and *deltex3*. In silico domain analysis revealed structural similarities as well as differences among the zebrafish *deltex* homologues. The most interesting difference is the lack of a WWE domain in Deltex3, which indicates that it may not interact with Notch receptors directly. Whole mount in situ hybridization assay demonstrated zebrafish *deltex1* expression in many tissues, including neural and sensory structures, raising the possibility that it may be involved in neurogenesis via the Notch signaling pathway. Increasing evidence suggests that Deltex possesses an E3 ligase activity and is responsible for endosomal trafficking of Notch through interaction with the Notch ankyrin repeats. The E3 ligase activity of zebrafish Deltex1 was carried out by in vitro ubiquitylation assay. Our result confirms that Deltex1 has an E3 autoligase activity in the presence of an E2, UbcH5a. Our characterization provides the first description of expression pattern of Deltex homologue and demonstration of its E3 ligase activity in zebrafish that would help in determining the molecular function of Deltex in the context of Notch signaling.

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### A zebrafish Pax6a reporter BAC recapitulates Pax6 expression in the mouse

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Pax6 is a highly conserved transcription factor which is crucial to the development of the central nervous system, eye and pancreas. Pax6 transcription is complex and subject to very strict regulatory mechanisms, which include a large number of tissue specific regulatory elements as well as a differential promoter usage. Unlike most vertebrates, zebrafish have two Pax6 genes, designated Pax6a and Pax6b, which are located on different chromosomes, and likely arose from a genome duplication that occurred after the split between the tetrapod and teleost lineages. The Pax6 proteins encoded by Pax6a and Pax6b share 95% amino acid identity over their entire length and both generate ectopic eyes in *Drosophila* suggesting that the two proteins have retained similar biochemical functions. Furthermore, it has been postulated that both genes have been retained due to a partitioning of certain tissue specific, regulatory elements, crucial to proper Pax6 function. In order to test the degree of evolutionary conservation of the mechanisms governing Pax6 expression as well as to further investigate the basis for the retention of two Pax6 genes in fish, we took advantage of BAC modification technology. We have tested a dual reporter BAC containing the zebrafish Pax6a transcription unit (200 kb) in both mouse and zebrafish and demonstrate that the teleost regulatory elements can in fact direct reporter gene expression in the mouse. Our findings directly show that the factors occupying distinct regulatory elements can induce proper Pax6 transcription despite the vast